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CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE PETROLEUM ETHER SOLUBLE MATERIAL OF FRESH AND CANNED FLORIDA VALENCIA ORANGE JUICE[1]

by
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Orange juice preserved by canning deteriorates in flavor during storage. The reason for this deterioration is not known with certainty, but it is generally agreed by authorities that such off-flavors are due in part to oxidation. The newer technique of deaeration and high-short pasteurization as compared with older methods of processing has greatly improved the quality of canned orange juice, but, nevertheless, after several months' storage at ordinary temperatures off-flavors will develop.

Nelson, Mottern, and Eddy (1933), believing that the nitrogenous constituents of the juice, which amount to about one percent of the total solids, might throw some light on the cause of the flavor deterioration undertook a study of the nitrogen bodies of fresh and aged, canned orange juice. No changes were detected in the nature of the constituents present in the deteriorated juice from those in fresh juice.

These authors point out that it is highly probable the development of off-flavors is due to some constituent or group of constituents present in small amounts. The fatty matter of orange juice, alleged to be present in amounts averaging about .2 percent according to Chatfield and McLaughlin (1931), might fall within this category. It was thought that off-flavor development may be caused by rancidity changes of this fat. The literature contains no references relative to the investigation of the fatty material of orange juice. Matlack (1929) has, however, made a study of the non-volatile constituents of the rind of (California) oranges. It is a well-known fact among whisky distillers that an extremely small amount of rancid fat in whisky will impart a disagreeable odor to the product.

In order to verify the theory that rancidity may cause off-flavors in canned orange juice, preliminary tests were conducted in samples of fresh juice and canned juice seven months old, which had a distinct off-flavor. The samples were extracted with ethyl ether, the ether solution was washed with water, and the extracts, after removal of solvent, were examined organoleptically. The fresh-juice extracts had a bland taste and fragrant odor, while extracts from the canned juice had an unpleasant, rancid taste and a stale, tallowy odor. Taufel and Thaler's (1932) ketone test when applied to the distillate from canned juice gave positive results, while a negative test was obtained in a distillate from fresh juice. Ketone rancidity can be detected by taste when methyl nonyl ketone or methyl heptyl ketone are present to the extent of only four parts per million, Schmalfuss, Werner, and Gehrke (1933).

Experimental Procedure

Since preliminary investigations indicated that rancidity actually occurred in aged, canned orange juice, a more detailed investigation of the problem seemed warranted.

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Florida Valencia oranges were used and the juice extracted by reaming them over slowly revolving burrs. This method of extraction is comparable to that now being used commercially. The juice used for investigation of the fresh fatty material had a Brix of 9.6°, a total acidity of 1.10 percent as anhydrous citric acid, and a pH of 3.82. The juice used for canning had a Brix of 8.8 to 11°, total acidity of .96 to 1.29 percent, and a pH of 3.69 to 3.53.

The juice was canned by deaerating, using a Pulley (1936) deaerator, screening through a 20-mesh screen, and pasteurizing at 90.5°C. (195°F.) for five seconds in a tin-coil pasteurizer, quickly cooling to 85°C. (185°F.), and filling into plain cans at this temperature -- Von Loesecke, Mottern, and Pulley (1934). The sealed cans were rapidly cooled and stored for 16 months at a temperature of 26.7 to 40.5°C. (80 to 105°F.).

Extraction of Fatty Material: One hundred forty-two liters (approximately 37.5 gallons) of the freshly reamed juice was immediately centrifuged four times by means of a Sharples Super-centrifuge, and the yellow, pasty material, which deposited in the centrifuge bowl, was collected. Even though the juice was centrifuged four times it still possessed a light-yellow color which would indicate that some extractive matter was not removed. To remove all of the extractive matter would have required more time than the slight additional yield would justify.

The thick, pasty material obtained was extracted several times with acetone[2] and then about 40 times with petroleum ether. The acetone extracts were evaporated to dryness and dissolved in petroleum ether. The petroleum ether extracts were combined, partially evaporated, filtered, and washed seven times with distilled water. The precipitate obtained consisted of about .4 gram of a waxy substance.

The washed petroleum ether extract was evaporated in vacuo and heated in vacuo at 80°C. (176°F.) until all solvent had been removed. Thirty-five grams (amounting to a yield of .024 percent based on the original juice) of a reddish brown solid of buttery consistency were obtained.

One hundred twenty-two liters (approximately 32 gallons) of the 16 months' oil canned juice were centrifuged and extracted in the same manner as described above for fresh juice. Fifty grams (amounting to a yield of .034 percent based on the weight of the canned juice) of a reddish-brown, thick liquid was obtained.

Examiration of Petroleum Ether Soluble Material: The extract obtained from fresh juice had a sweet, fragrant odor and a bland, oily taste; that obtained from the aged, canned juice possessed a stale odor and a rancid, bitter taste. The extract from fresh juice was a buttery solid at room temperatures; while that from aged, canned juice was a thick liquid at room temperatures (Table 1).

Saponification of Fat and Separation of Saturated and Unsaturated Acids: To a weighed portion of the extract from fresh juice was added an excess of .5N alcoholic potassium hydroxide and the mixture was refluxed for four hours. The alcohol was removed by distillation, water was added, and the soaps extracted seven times with petroleum ether; this extract was reserved for examination of

2. Acetone was first used to partially remove the water and hence make easier subsequent extractions with petroleum ether.

unsaponifiable constituents.

The aqueous soap solution was acidified with dilute hydrochloric acid and the mixture extracted seven times with petroleum ether. The acid layer was observed to contain a reddish-brown solid and was reserved for future examination of resins and phytosterolines. The petroleum ether was washed, the wash waters being added to the acid layer above, and the petroleum ether removed by distillation. The residue obtained was dried at 100°C. (212°F.) under a blanket of carbon dioxide. It contained 36.9 percent solids composed chiefly of free fatty acids. The residue was taken up in alcohol, a slight excess of alcoholic potash was added, and the separation of the saturated and unsaturated fatty acids was carried out by the lead salt-ether method. The total free fatty acids obtained had an iodine number of 111.2 and amounted to 31.5 percent of the total solids, the difference between this figure and 36.9 percent obtained above being considered as resinous material.

Because of the profound change that had taken place in the characteristics of the extract from the aged, canned juice, separation of unsaturated and saturated acids could not be satisfactorily accomplished by the lead salt-ether method. The oxidized extract contained appreciable quantities of hydroxy acids (shown by the high apparent acetyl value, Table 1) which are readily converted to lactones when subjected to the treatment called for in the lead salt-ether method. Lactones, being neutral compounds, show an apparent decrease in neutralization values. Since lactones are readily soluble in ether and alcohol, they affect the separation of the fatty acids from the resins causing an apparent increase in the fatty-acid fraction and a decrease in the resin fraction. The values obtained are, however, significant in that the differences between the saponification and neutralization values are due to the hydroxy acids becoming dehydrated and forming inner anhydrides, characteristic in the oxidation of fatty acids, Lewkowitsch (1921a). In spite of this, the lead salt-ether method was used for the separation of the unsaturated and saturated fatty acids in aged, canned juice. The total free fatty acids amounted to 44.9 percent, a higher value than obtained for the fresh juice because of the presence of resins and lactones. Characteristics of the free fatty acids from fresh juice and aged, canned juice are given (Table 2).

The constants obtained for the free fatty acids in aged, canned juice show that separation was not clear-cut and confirms the hypothesis regarding the apparent presence of lactones.

Further attempts were made to obtain a clean separation of the acids by converting the petroleum ether soluble material from the aged, canned juice into ethyl esters. The esters were diluted with water, heated to boiling, cooled, and extracted with petroleum ether. The petroleum ether was washed with water and then treated with .2N potassium hydroxide and a small amount of alcohol. The petroleum ether solution of the esters was floating on top of a watery layer of resin soaps. The esters were washed with water, excess solvent was removed by distillation, and the residue was dried at 100°C. in a blanket of carbon dioxide. The dried esters were then extracted with methyl alcohol and filtered. The methyl alcohol extract was acidified with hydrochloric acid and treated with petroleum ether. The petroleum ether extract was washed free of acids, the solvent was removed by distillation, and the residue was dried at 100°C. under carbon dioxide. The constants obtained for the fatty acids separated by this method were essentially the same as those obtained for the fatty

acids separated by the conventional lead salt-ether method. It is therefore concluded that the method described is no more efficient than the lead salt-ether method.

Composition of Unsaturated Acids: The iodine number of the unsaturated acids of fresh juice was 118.5. When aliquot portions of an ether solution of the unsaturated acids of fresh juice were brominated in the cold, no hexabromide precipitate was observed. This fact proved the absence of not more than faint traces of linolenic acid. Since, therefore, the unsaturated acids consisted essentially of only oleic and linoleic acid, the percentage of these acids was calculated according to Lewkowitsch (1921b); 30 percent of linoleic acid and 70 percent of oleic acid were obtained.

As previously stated, a clean separation of the unsaturated and saturated acids could not be obtained in the case of aged, canned juice. It was therefore impossible to identify the unsaturated acids.

Composition of Saturated Acids: The iodine number of the saturated acids from fresh juice was 6.33. Insufficient material was available to attempt a quantitative separation of the saturated acids of the fresh juice. Identification was, however, attempted, by recrystallizing the mixed acids from alcohol-acetone solutions. An acid was obtained melting between 82 and 85°C. (179.6 and 185°F.). The acid was converted into its potassium salt which was found to be insoluble in cold water. Since the potassium salt of cerotic acid is insoluble in cold water, this fact, together with the melting point of the acid, indicates that this factor is composed chiefly of cerotic acid.

Further crystallizations of the mother liquor obtained after removal of cerotic acid yielded a crystalline acid melting between 65 and 69°C. (149 and 156.2°F.). Stearic acid melts between 69 and 71°C.; (156.2 and 159.8°F.); palmitic acid, between 62.6 and 63°C. (144.6 and 145.4°F.). The crystals obtained were apparently a mixture of these two acids, consisting chiefly of stearic acid.

The mother liquor from these crystallizations was evaporated to dryness, the crystals obtained melting between 56 and 59°C. (132.8 and 138.2°F.). This was considered to be a mixture of stearic and palmitic acid, chiefly the latter.

Although the saturated acids obtained from the aged, canned juice were impure because of reasons previously stated, attempts were made to identify the acids by repeated recrystallization from ethyl alcohol. Two fractions were obtained -- one melting at 73 to 75°C. (163.4 to 167°F.) and the other at 53 to 55°D. (127.4 to 131°F.). Their molecular weights were calculated from the neutralization value, but they did not correspond to the molecular weights of any known fatty acid. It is probable these acids were so grossly contaminated with lactones and resins, their clean separation would be virtually impossible.

Extraction of Resins: This fraction has been called resins because of the solubility of the material in various solvents, and its saponification, iodine, and acid values, all of which are within the range of substances classified as resins.

The liquid containing reddish-brown material after the extraction of the fatty acids from fresh juice with petroleum ether was extracted with isoamyl

alcohol. The alcohol was washed several times with water, the solvent removed by distillation, and the residue dried at 100°C. (212°F.). A dark-red solid of buttery consistency was obtained, equivalent to 53.8 percent of the total solids. When fused with potassium hydroxide, negative results were obtained for phenols. No nitrogen was found present.

The characteristics of the resins obtained from the aged, canned juice were quite different from those found in fresh juice (Table 3).

Inasmuch as the resins obtained from aged, canned juice contain lactones resulting from the chemical treatment afforded the hydroxy acids during separation, as explained above under the discussion of the separation of the free fatty acids, they are not true values. The percentage of resins in the aged, canned juice appears to be less than it actually is because a portion of the resins was extracted and included in the free fatty-acid fraction owing to the solubility of the lactones in petroleum ether. Thus, the percentage of free fatty acids in the petroleum ether extract from fresh juice was 31.5 percent, while from aged, canned juice it was 44.9 percent.

Isolation of Phytosterolines: A portion of the resinous material from fresh juice was shaken with an excess of .5N sodium hydroxide and the mixture allowed to stand until the solids had disintegrated. The mixture was filtered, the precipitate washed with water until free of alkali, dried and weighed. The phytosterolines thus obtained amounted to .3 percent, based on the weight of the petroleum ether extract. The presence of phytosterolines was confirmed by refluxing with amyl alcohol and dilute hydrochloric acid, neutralizing with sodium carbonate, and boiling with Fehling's solution. The Liebermann-Burchard reaction for sterols was also positive.

The same procedure as above was carried out on the resinous material from aged, canned juice, .4 percent being obtained. Considering that separation is not strictly quantitative, it can be said there is no difference in the phytosteroline content of fresh and aged, canned juice.

Insufficient material prevented positive identification of these phytosterolines.

Determination of Carotinoid Pigments: Carotinoid pigments were determined according to the colormetric method of Sprague (1928), using napthol yellow and orange G as a standard. The petroleum ether soluble extract of fresh juice contained .11 percent carotinoid pigments.

Isolation of Phytosterols: The unsaponifiable material obtained from the soap solution by extraction with petroleum ether amounted to 5.04 percent of the total extract in the case of fresh juice, and to 1.77 percent of the total extract in the case of aged, canned juice.

A one-percent alcoholic solution of digitonin was added to the unsaponifiable matter, the solution shaken, warmed, and allowed to stand overnight. Ethyl ether was added to the alcoholic solution, the precipitate filtered off, washed first with alcohol and then with ether, dried, and weighed.

The phytosterol equivalent of the digitonide obtained was approximate .2 percent of the total extract for both fresh and aged, canned juice.

The presence of sterols was confirmed by the Liebermann-Burchard reaction.

The filtrate from the digitonite was evaporated to remove the solvent, acetic anhydride added, and the mixture refluxed. The material dissolved completely in the hot acetic anhydride, indicating the presence of aliphatic alcohols. The acetylated mass was poured into boiling water to hydrolyze the excess acetic anhydride, allowed to cool, and the precipitate was filtered, washed free of acid, and dried. The acetate of the higher alcohols thus obtained amounted to .48 percent of the total extract in the case of fresh juice. In the case of the extract from aged, canned juice, results were so erratic they were considered unreliable.

The quantity of the acetates was too small to attempt identification.

Discussion of Results

The petroleum ether extract obtained from fresh orange juice had a bland taste and aromatic odor, while that from aged, canned juice had a stale, tallowy odor and a rancid taste with bitter after-taste.

The fatty material of orange juice undergoes profound changes when the juice is canned and stored at elevated temperatures. That such changes are primarily brought about by oxidation is shown by the fact that the refractive index, iodine number, and insoluble acids decreased; while there was an increase in acidity and saponification value. There was an increase from .006 to .30 in the peroxide value (M). Whereas the fatty material from fresh juice showed no Kreis test, that obtained from aged, canned juice showed a positive reaction.

It will be noted too, that the extraction from aged, canned juice indicated higher ketones. This is significant in that minute amounts of the higher ketones can be detected by their unpleasant taste. The presence of these higher ketones was not only established in the extract of the fatty material but also in a distillate from aged, canned juice.

An increase in the Issoglio oxidizability number of the extract from aged, canned juice indicates that oxidation created volatile decomposition products.

The aldehyde test was negative in the case of the rancid extract of the aged, canned juice. This may be explained by the fact that when a fat is oxidized in the presence of moisture, acids are the final products. In fact, extreme oxidation of fats is characterized by the absence of aldehydes.

The high acetyl value of the fatty material from aged, canned orange juice indicates that the oxidized fats and resins have been partly changed to hydroxy acids.

From the above facts it is quite apparent that the fatty material in orange juice undergoes oxidative changes when such juice is canned according to present methods and subjected to storage. Since it has been shown that rancidity results in a change of flavor of the fat and the creation of oxidative products, it is quite reasonable to suppose this phenomenon would result in off-flavors in the canned juice. Whether or not oxidation of the fatty material is entirely responsible for deterioration in flavor of canned orange juice cannot be stated

in the light of the evidence presented here. It is, however, reasonable to assume that such oxidation of the fatty material assumes an important role in the development of off-flavors.

The data do not indicate how these oxidative changes are brought about, but they may result through oxygen left in the headspace of the container[3] or through enzyme activity. During the process of extracting the juice it is possible, too, that the fat in the juice absorbs oxygen to form loosely bound oxygen compounds, which Holm, Greenbank, and Deysher (1927) have called "moloxides". They are alleged to be responsible for oxidation even in vacuo.

Summary

The petroleum ether soluble matter of fresh and aged, canned orange juice has been investigated. In fresh juice this consists of unidentified waxy bodies insoluble in petroleum ether; oleic, linoleic, cerotic, palmitic, and stearic acids; unidentified aliphatic alcohols and resins; phytosterols; sterols; and carotinoid pigments.

The characteristics of the petroleum ether extract of aged, canned orange juice indicated that the fatty material had undergone oxidative changes, with the creation of hydroxy acids and other decomposition products, and had become rancid.

In view of the evidence presented it is believed that off-flavors subsequently developing in canned orange juice are, to some extent, due to the oxidation of the fat in the juice.

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Table I - Characteristics of Petroleum Ether Extract of Fresh and Aged, Canned Florida Valencia Orange Juice

	Fresh Juice	Aged, canned Juice
Index of refraction $N_d 20^\circ C.$	1.5078	1.4825
Acid value	23.5	77.4
Iodine No. (Hanus)	87.9	73.7
Saponification value	142.8	183.8
Insoluble acids (Hehner)	76.3	58.0
Peroxide value (M)	0.006	.30
Kreis test	Negative	Positive
Higher ketone test (Taufel)	Negative	Positive
Issoglio oxidizability number	4.5	13.8
Aldehyde test (Schibstead)	1.7	Nil
Apparent acetyl value	14.9	125.1

Table 2 - Characteristics of Free Fatty Acids of Fresh and Aged, Canned Florida Valencia Orange Juice

	Fresh Juice	Aged, Canned Juice
Neutralization number	173.4	152.5
	pct.	pct.
Saturated acids (corrected)	6.90	13.43
Unsaturated acids (corrected)	78.40	57.28

Table 3 - Constants of Resins Separated from Fresh and Aged, Canned Florida Valencia Orange Juice

	Fresh Juice	Aged, Canned Juice
Percent of resin[1]	53.8	36.4[2]
Acid number	33.9	93.5
Saponification number	72.1	126.6

1. Based on total petroleum ether extract
2. Apparent percentage

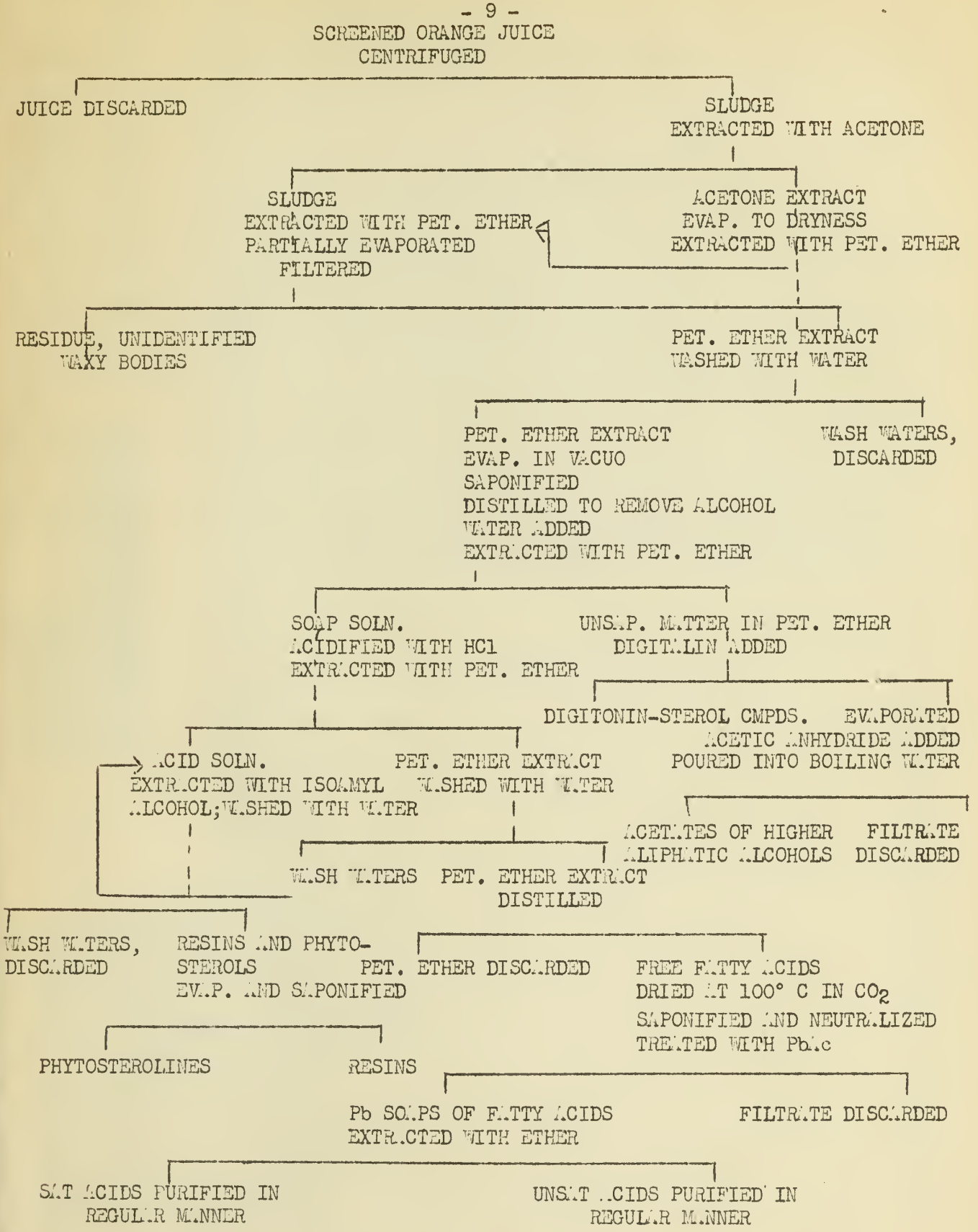

FIG. 1. Diagrammatic outline for the separation of the petroleum ether soluble constituents of orange juice.

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